Presumptive Color Tests for GHB

Introduction:

There are three presumptive color tests that may be used to screen a sample for the presence of Gamma-hydroxybutyric Acid (GHB) that have been approved for use by this laboratory. It should be noted that it is not necessary to perform all three tests, however more information will be derived if all three of these tests are performed.

Additionally, the color of the sample solution should be taken into account when evaluating the color changes that may occur.

Safety:

All safety requirements as outlined in the Unit and Lab-wide safety manuals should be followed. Note that some of the materials may be toxic.

Materials Needed to Prepare Reagents:

Methanol
Water
Ethanol
Dextrose
Aniline Hydrochloride
Chlorophenol Red
Bromocresol Purple
Bromothymol Blue
Bromocresol Green
Methyl Orange
Gamma-hydroxybutyric Acid
10%NaOH,

GHB Spot Test #1:

Reagent Preparation:
(Note: The total reagent volume may be adjusted as needed, as long as the ratios of materials is consistent as described below).

1. Chlorophenol Red:
   0.004 grams chlorophenol red in 10 ml water, adjust pH to 7.0 with 0.1N NaOH.

2. Modified Schweppes Reagent
   Solution A: 0.50 grams dextrose in 5 ml water
   Solution B: 0.60 grams aniline hydrochloride in 5 ml ethanol.
   Mix both solutions together and dilute to 20 ml total volume with methanol.

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GHB Test #1:
Test Reagent: Mixture of 3 ml Chlorphenol red and 1 ml Modified Schweppes Reagent.

A small amount of sample in liquid is added to a culture tube. The pH of this solution should be checked and recorded. If necessary, the pH should be adjusted with 0.1N NaOH to 7. Then, 1-2 drops of test reagent #1 is added to the culture tube and gently mixed. Additionally, the test reagent should be tested on distilled water to check the effectiveness of the reagent.

A positive result will produce a color between orange and dark red, depending on the concentration of the sample. An immediate orange-red color indicates the presence of GHB.

The distilled water (negative control) should produce a yellow color, similar to the color of the reagent. If this does not occur, then the reagent should be discarded.

A known standard of GHB should be run as well. An immediate orange-red color indicates a positive result.

GHB Spot Test #2:

Reagent Preparation:
(Note: The total reagent volume may be adjusted as needed, as long as the ratio of materials is consistent as described below).

1. Bromocresol Purple:
   0.004 grams bromocresol purple in 10 ml water, adjust pH to 7.0 with 0.1N NaOH.

2. Bromothymol Blue:
   0.004 grams bromothymol blue in 10 ml water, adjust pH to 7.0 with 0.1N NaOH.

3. Modified Schweppes Reagent
   Solution A: 0.50 grams dextrose in 5 ml water
   Solution B: 0.60 grams aniline hydrochloride in 5 ml ethanol.
   Mix both solutions together and dilute to 20 ml total volume with methanol.

GHB Test #2:
Test Reagent: Mixture of 4 ml of bromocresol purple and 4 ml of bromothymol blue. Then, 7 ml of this mixture is added to 1 ml of Modified Schweppes Reagent and mixed.

A small amount of sample in liquid is added to a culture tube. The pH of this solution should be checked and recorded. If necessary, the pH should be adjusted with 0.1N NaOH to 7. Then, 1-2 drops of test reagent #2 is added to the culture tube and gently mixed. Additionally, the test reagent should be tested on distilled water to check the effectiveness of the reagent.

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A positive result will produce a color between lavender and deep purple, depending on the concentration of the sample. An immediate purple color indicates the presence of GHB.

The distilled water (negative control) should produce a yellow color, similar to the color of the reagent. If this does not occur, then the reagent should be discarded.

A known standard of GHB should be run as well. An immediate lavender/deep purple color indicates a positive result.

GHB Spot Test #3:

Reagent Preparation:
(Note: The total reagent volume may be adjusted as needed, as long as the ratio of materials is consistent as described below).

1. Bromocresol Green:
   0.003 grams bromocresol green in 10 ml water, adjust pH to 7.0 with 0.1N NaOH.

2. Methyl Orange:
   0.01 grams methyl orange in 10 ml water, adjust pH to 7.0 with 0.1N NaOH.

3. Modified Schweppes Reagent
   Solution A: 0.50 grams dextrose in 5 ml water
   Solution B: 0.60 grams aniline hydrochloride in 5 ml ethanol.

Mix both solutions together and dilute to 20 ml total volume with methanol

GHB Test #3:
Test Reagent: Mixture of 4 ml of bromocresol green and 4 ml of methyl orange. Then, 6 ml of this mixture was added to 2 ml of Modified Schweppes Reagent and mixed.

A small amount of sample in liquid is added to a culture tube. The pH of this solution should be checked and recorded. If necessary, the pH should be adjusted with 0.1N NaOH to 7. Then, 1-2 drops of test reagent #2 is added to the culture tube and gently mixed. Additionally, the test reagent should be tested on distilled water to check the effectiveness of the reagent.

A positive result will produce a color between leaf green and deep blue-green, depending on the concentration of the sample. An immediate green color indicates the presence of GHB.

The distilled water (negative control) should produce an orange-pink color, similar to the color of the reagent. If this does not occur, then the reagent should be discarded.

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A known standard of GHB should be run as well. An immediate leaf-green/deep blue-green color indicates a positive result.

Quality Control Procedure of Reagents:
All of the guidelines outlined in the Drug Unit Quality Control Manual will apply to these GHB Reagents, with this additional information:

The code designation for solution lot numbers will be assigned from the following list:
GHB1=Test Reagent 1
GHB2=Test Reagent 2
GHB3=Test Reagent 3
As indicated, all of the lot numbers will begin with number 101 and be assigned consecutively (eg. GHB1-101, etc)

The reagents do not need to be tested on a regular schedule. However, each time they are used a positive and a negative control should be run to insure they are working properly (see procedure description). The reagents should be stored in the refrigerator when not in use.

Reference:
Microgram, Volume XXXV, No. 1, January 2002

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